Amendments to the Specification

(1) Please replace the paragraph on page 5, lines 8-9, with the following paragraph:

B2

Figure 5 shows the result of GeneChip® sequence analysis of p53 genes in normal and malignant breast epithelium cells. The genotype analysis shows a G (see 1209_B01.01 and et121701.02 (SEQ ID NO:3)) to A (see et121702.02 (SEQ ID NO:5)) base change resulting in a E (see 1209_B01.01 and et121701.02 (SEQ ID NO:4)) to K (see et121702.02 (SEQ ID NO:6)) amino acid change at position 285 in exon 8, the p53 DNA binding domain.

(2) Please delete the paragraph on page 6 at lines 1-2.

(3) Please replace the paragraph on page 6, lines 3-21 with the following paragraph:

B3

Figure 8 illustrates ~~Figures 9A and 9B illustrate~~ fluorescence images of oligonucleotide arrays monitoring 1,650 genes in parallel (1 of a set of 4 arrays covering 6,600 genes). ~~In Fig. 9A representative~~ Representative hybridization patterns of fluorescently labeled cRNA from normal (HT-125) and malignant breast (BT-474) cells are shown. The images were obtained after hybridization of arrays with fragmented, biotin labeled cRNA and subsequent staining with a phycoerytherin-strepavidin conjugate. Bright rows indicate messages present at high levels. Low level messages (1-10 copies/cell) are unambiguously detected based on quantitative analysis of PM/MM intensity patterns. In the ~~lower~~ middle portion of the Figure a magnified view of a portion of the array highlighting examples of altered gene expression between BT-474 and HT-125 is shown. In area 1, induced (>10-fold change in hybridization intensity) genes are shown, in area 2, unchanged (<2-fold

change in hybridization intensity) are shown, and in area 3 repressed (>10-fold change in hybridization intensity) are shown. ~~Fig. 9B illustrates zoom-in~~ Zoom-in images of genes 1, 2, & 3 in ~~(A)~~ the inset, above, are illustrated as 20 probe pairs of perfect-matched (PM) and single base mis-matched (MM) oligonucleotide probe cells. Average ~~fluoresence~~ fluorescence intensity difference for PM-MM in HT-125 versus BT-474 (normalized to β-Actin and GAPDH signals) are shown in the three rows. In row 1 (Her2/neu oncogene) the average intensities are 111 versus 5,127; in row 2 (laminin receptor) 3,495 versus 6,088; and in row 3 (galectin-1) 7,952 versus undetected.

(4) Please replace the paragraph on page 6, lines 22-26, with the following paragraph:

Figure 9A and B ~~10~~ illustrates expression profiles of subset of genes from normal (Figure 9A) versus malignant (Figure 9B) breast cells. Average perfect match-mismatch ~~(PM-MM)intensity~~ (PM-MM) intensity differences (normalized to β-Actin and GAPDH signals) were plotted for the genes highlighted in Figure ~~9A~~ 8 that demonstrated greater than a 2-fold difference in hybridization signals between HT-125 and BT-474. Values for signals off scale are indicated.

(5) Please replace the paragraph on page 6, line 27 to page 7, line 11 with the following paragraph:

Figure ~~11 illustrates~~ 10A, B, and C illustrates p53 sequence analysis and mutation detection by hybridization. In Figure ~~11A~~ 10A, an image of the p53 genotyping array hybridized to 1,490 bp of the BT-474 breast carcinoma p53 gene (left) is shown. A zoom-in view of hybridization patterns of p53 wild-type reference and BT-474 DNA in a region

B3

of a G→A single-base mutation in BT-474 is shown at the right. In each column are 4 identical probes with an A, C, G or T substituted at a central position. The hybridized target sequence identified based on mismatch detection from left to right as the complement of the substitution base with the brightest signal. The G→A transition seen in BT-474 is accompanied by a loss of signal at flanking positions as these probes now have a single-base mismatch to the target distinct from the query position. Fig. ~~11B~~ 10B (top), comparison of wild-type reference (black) and BT-474 p53 gene (red) hybridization intensity patterns from sense (above) and anti-sense strands (below; Fig. 10C) in the region containing a mutation. The area shown demonstrates the "footprint" and detection of a single-base difference between the samples (vertical green line). GeneChip data analysis output is shown (bottom) that unambiguously identifies a G→A base change at nucleotide 1,279 of p53 in BT-474 resulting in a glutamic acid to lysine amino acid change in exon 8 (DNA binding domain). The upper portion of output displays the p53 wild-type reference nucleotide (SEQ ID NO:3) and amino acid (SEQ ID NO:4) sequence. Aligned sequence outputs of wild-type p53 control nucleotide (SEQ ID NO:3) and amino acid (SEQ ID NO:4) and BT-474 nucleotide (SEQ ID NO:5) and amino acid (SEQ ID NO:6) samples are shown.

~~(6)~~ Please add the following new paragraph immediately following the paragraph ending on page 7, line 11:

Figure 11A-M is a table that shows the genes transcriptionally induced in BT-474 vs. HT-125 cells.

B3 Cancel

(7) Please replace the paragraph at page 40, lines 1-13 with the following paragraph:

B4

The purpose of statistical analysis is to establish and test causal models for the genetic network. It is apparent to those skilled in the art that those causal models can be used to generate dynamic maps of regulatory pathways. A variety of statistical methods that have been developed for understanding complex systems are useful for some of the embodiments. In some embodiments, cluster analysis methods are used to group genes whose expression level is correlated. Methods for cluster analysis are described in detail in Harfigan (1975) Clustering Álgorithms, NY, John Wile and Sons, Inc, and Everritt, (1980) Cluster Analysis 2nd. Ed. London Heineman Educational books, Ltd., incorporated herein for all ~~purposed~~ purposes by reference. The causal relationships in a genetic network can also be modeled by stochastic procedures. Such models allow the examination of the dynamical aspects of the genetic network in terms of change over time or across conditions. Maybeck, Stochastic Models, estimation and control, vol. 1, (1979) NY, Academic Press.

(8) Please replace the paragraph at page 43, lines 6-11 with the following paragraph:

B5

Approximately 65,000 unique DNA probes (10^7 unique probes/50 mm^2 area) were synthesized on a 1.2 cm^2 glass slide. A set of 4 different oligonucleotide arrays that include more than 6,500 human gene sequences derived from the GenBank (~~http://www.ncbi.nlm.nih.gov~~ URL address: http file type, www host server, ncbi.nlm.nih.gov domain name) and dbEST databases were generated. These arrays were used to monitor and compare the expression of >6,500 genes in parallel from normal and malignant breast tissue cell lines.

(9) Please replace the paragraph at page 47, lines 1-6 with the following paragraph:

Approximately 65,000 unique DNA probes (10^7 unique probes/50 mm^2 area) were synthesized on a 1.2 cm^2 glass slide. A set of 4 different oligonucleotide arrays that include more than 6,500 human gene sequences derived from the GenBank (~~http://www.ncbi.nlm.nih.gov~~ URL address: http file type, www host server, ncbi.nlm.nih.gov domain name) and dbEST databases were generated. These arrays were used to monitor and compare the expression of >6,500 genes in parallel from normal and malignant breast tissue cell lines.

(10) Please replace the paragraph at page 58, lines 1-19 with the following paragraph:

To investigate the cause of the transcriptionally inactive p53, genomic p53 was resequenced in BT474. The strategy for rapid, simultaneous analysis of large amounts of genetic information using high-density oligonucleotide arrays has been described in Chee, M., Yang, R., Hubbell, E., Berno, A., Huang, X.C., Stern, D., Winkler, J., Lockhart, D.J., Morris, M.S. & Fodor, S.P. Accessing genetic information with high-density DNA arrays, *Science* **5287**, 610-614 (1996). The DNA array used in this study allowed for simultaneous analysis of both sense and anti-sense sequence of p53 coding exons 2-11, including 10 base pairs of intronic flanking sequence (to identify splice donor-acceptor mutations), as well as allele specific probes for over 300 characterized hotspot p53 mutations and every possible single base deletion (Dee et al., manuscript in prep.) The re-sequence analysis portion of the DNA array consisted of a set of 4 identical 20-mer oligonucleotides complementary to p53 wild-type sequence, except that an A,C,G or T was substituted into each probe at a centrally localized position. In each set of four probes, the

perfect complement to the target sequence will hybridize more strongly than the single base mismatched probes (see Fig. ~~3A~~ 10A, wild-type), allowing unambiguous sequence assignment by automated basecalling software (30). By ~~usingsets~~ using sets of 4 probes in this manner that span 1,490 bp of the p53 sequence, a single person can fully genotype the p53 gene from 60 genomes in the time it would take to do 12 by conventional gel-based de novo sequencing.

(11) Please replace the paragraph at page 58, lines 20-29 with the following paragraph:

To facilitate characterization of mutations in the p53 gene we applied an algorithm that performs base identification of nucleotide changes between a sample and a reference. This sequence analysis is based on two major effects that a single base change has on the array hybridization pattern of an experimental sample relative to a wild-type reference: 1) The probe containing the substitution base displays the strongest signal of the 4 probe set; and 2) The neighboring probes that overlap the position display a characteristic loss of signal or "footprint" for probes flanking a base substitution, as these probes would have a single base mismatch to the mutated target sequence distinct from the query base (see Fig ~~3A~~ 10A, BT-474 versus wild-type, and ref. 30).

(12) Please replace the paragraph at page 58, line 30 to page 59, line 10 with the following paragraph.

The analysis of BT-474 versus HT-125 p53 genomic DNA using the p53 genotyping array revealed a single base substitution of G to A in exon 8 (DNA binding domain), resulting in an amino acid change at position 285 from E to K (Fig. ~~3B~~ 10B).

B7

The hybridization signal difference centered about the mutation identified by the footprint analysis (see Fig. ~~3B~~ 10B, top panel), and subsequent base calling of a single genotype (see Fig. ~~3B, bottom panel~~ 10C) in BT-474 by the GeneChip software indicated that this carcinoma had a loss of heterozygosity at the p53 locus (confirmed by dideoxy sequence analysis). These data unambiguously show that the loss of wild-type p53 transcriptional function in these cells was due to the absence of wild-type p53 protein. We have applied this analysis to other breast carcinomas with similar outcomes correlating altered gene expression patterns of targets of p53 transcriptional modulation with mutant p53 gene status (Table 3).

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(13) Please replace the paragraph on page 60, line 28 to page 61, line 22 with the following paragraph:

Gene expression array hybridization and scanning. 10 μg of biotinylated cRNA target was fragmented to an average size of 50 nucleotides in 10 μl of magnesium fragmentation buffer (40mM Tris-acetate (pH 8.1), 100mM KOAc, 30mM MgOAc) at 95°C for 35 min. The fragmented samples were brought up to a final volume of 200 μl with hybridization buffer (0.9 M NaCl, 60 mM NaH_2PO_4, 6 mM EDTA and 0.005% Triton X-100, pH 7.6 (6xSSPE-T)) containing 0.1 ng/ml Herring Sperm DNA, 50 pM biotin-labeled control oligo (5'-GTCAAGATGCTACCGTTCAG-3' (SEQ ID NO:1)) and biotinylated cRNA quantitation standards bioB (1.5 pM), bioC (5.0 pM), bioD (25 pM) and Cre (100 pM). Samples were denatured at 95°C for 10 min, chilled on ice for 5 min and equilibrated to room temperature (5 min) before being applied to the array flow cell. Arrays were hybridized at 40°C for 14-16 hr with rotation at 60 rpm, followed by 10 wash

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cycles (2 drain-fills/cycle) at room temperature with 6xSSPE-T in the GeneChip Fluidics Station (RELA). For staining of hybridized target cRNA, arrays were first washed in 0.5X SSPE-T at 40°C for 15 min with rotation (60 rpm), then incubated with 2 μg/ml of phycoerytherin-strepavidin conjugate (Molecular Probes) in 6xSSPE-T containing 1 mg/ml of acetylated-bovine serum albumin at 40°C for 10 min. Prior to scanning, the arrays were washed at room temperature with 6xSSPE-T for 5 cycles (2 drains-fills/cycle) in the fluidics station. The hybridized stained arrays were scanned using an argon-ion laser GeneChip scanner 50 (Molecular Dynamics) with a resolution setting of 7.5 μm/pixel (~45 pixels/probe cell), and wavelength detection setting of 560 nm. Fluorescence images and quantitative analysis of hybridization patterns and intensities were performed using GeneSeq Analysis Software and GEprocess (Affymetrix) gene expression data analysis programs.

B8 cont'd

(14) Please replace the paragraph on page 61, line 23 to page 62, line 7 with the following paragraph:

B9

p53 PCR and labeling for re-sequence analysis by array hybridization. The p53 gene was genotyped by amplifying coding exons 2-11 in a 100 μl multiplex PCR reaction using 100 ng of genomic DNA extracted from cells using a QIAmp Blood Kit (Qiagen). PCR Buffer II (Perkin-Elmer) was used at 1X along with 2.5mM $MgCl_2$, 200 μM of each dNTP and 10 units of Taq Polymerase Gold (Perkin-Elmer). The multiplex PCR was performed using 10 exon-specific primers ~~(Table 4)~~ with the following cycling conditions: 1 cycle at 94°C (5 min), 50 cycles of 94°C (30 sec), 60°C (30 sec) and 72°C (30 sec), followed by 1 cycle at 72°C (7 min). 45 μl of the PCR reaction was then

fragmented and dephosphorylated by incubation at 25°C for 15 min with 0.25 units of Amp Grade DNAse I (Gibco/BRL) and 2.5 units of Calf Alkaline Phosphatase (Gibco/BRL), followed by heat-inactivation at 99°C for 10 min. The fragmented PCR products were then labeled in a 100 µl reaction using 10 µM flourecein-N6-ddATP (Dupont-NEN) and 25 units of terminal transferase (Boehringer Mannheim) in 200 µM K-Cacodylate, 25 mM Tris-HCl (pH 6.6), 0.25 mg/ml BSA and 2.5 mM $CoCl_2$. The labeling reaction was incubated at 37°C for 45 min and heat-inactivated at 99°C for 5 min.

(15) Please replace the paragraph on page 62, lines 8-18 with the following paragraph:

p53 re-sequence analysis array hybridization and scanning. The fragmented, labeled PCR reaction was hybridized to the p53 re-sequence analysis array in 6xSSPE-T containing 2mg/ml BSA and 1.67 nM fluorescein-labeled control oligo (5'-CTGAACGGTAGCATCTTGAC-3' (SEQ ID NO:2)) at 45°C for 30 min. The array was then washed with 3X SSPE-T at 35°C for 4 cycles (10 drains-fills/cycle) in the GeneChip Fluidics Station (RELA). The hybridized p53 array was scanned using an argon-ion laser scanner (Hewlett-Packard) with a resolution setting of 6.0 µm/pixel (~70 pixels/probe cell) and wavelength detection setting of 530 nm. A fluorescence image was created, intensity information analyzed and nucleotide determination made by GeneChip Analysis Software (Affymetrix). Footprint analysis was done using Ulysses Analysis Software (Affymetrix) essentially as described.

(16) Please enter the paper copy of the Sequence Listing immediately following the claims.